Characterization of a Human Melanosome-Associated Antigen Recognized by Monoclonal Antibody, HMSA-2

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This study elucidates the nature of antigens recognized by monoclonal antibody (MoAb) HMSA-2, which was developed against human melanosome-associated antigen (HMSA) of malignant melanoma (Maeda and Jimbow, Cancer 59:415–423, 1987). Through flow cytometry analyses, indirect immunoprecipitation of antigen biosynthetically labeled with ^{35}S-methionine, enzyme-linked immunosorbent assays and immunoelectron microscopy, we found that a) the antigens recognized by MoAb HMSA-2 were melanosomal matrix glycoproteins; b) these antigens were expressed mainly in the cytoplasm, although they could also be detected on the cell surface; c) the cytoplasmic expression of MoAb HMSA-2 was cell-cycle dependent; d) large amounts of these antigens were released into culture supernatants; e) MoAb HMSA-2 immunoprecipitated two major glycoproteins with molecular weights of 94 and 53 kDa from culture supernatants, and f) both components have complex N-linked oligosaccharide chains with sialic acid, suggesting that these melanosomal proteins are derived from the transcisternae of the Golgi. These human melanosome-associated antigens may prove useful not only for studying the immunobiology of melanogenesis, but also for the immunodiagnosis of melanocytic disorders. *J Invest Dermatol 94:221–226, 1990*

The melanosome is a specific secretory organelle produced by melanocytes and malignant melanoma cells. It is composed of melanin, the melanin-forming enzyme, tyrosinase, structural matrix proteins, and phospholipids. To study further the immunobiology of human melanosomes, investigators have developed several monoclonal antibodies (MoAb) against partially purified melanosomal proteins of human malignant melanoma cells [1–3]. These antibodies reacted preferentially with sections of formalin-fixed and paraffin-embedded tissues [1–3]. The specificities and tissue distributions of the antigen(s) reactive with one of these antibodies, designated HMSA-2, were studied. This MoAb proved to be a useful diagnositc tool, particularly for amelanotic malignant melanoma and dysplastic melanocytic nevi [4,5].

The present study was aimed at 1) determining the level of HMSA-2 reactivity throughout the cell cycle; 2) examining the distribution of HMSA-2 reactive antigen in cultured human melanoma cells by immunoelectron microscopy; 3) determining whether the HMSA-2 antigen was shed into culture supernatants; 4) determining the biochemical characteristics of the HMSA-2 antigen shed into culture supernatants, and 5) comparing the immunologic specificity of HMSA-2 antigen with other cytoplasmic melanoma antigens. We have observed that the antigens recognized by MoAb HMSA-2 appear to be structural matrix glycoproteins, which are expressed mainly in the cytoplasm, but could be detected in smaller quantities on the cell surface. Large amounts of the antigens were released into supernatants of cultured human melanoma cells.

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Abbreviations:
ELISA: enzyme linked immunosorbent assay
FBS: fetal bovine serum
HMSA: human melanosome-associated antigen
HMW: high molecular weight
kDa: kilodalton
MAA: melanoma-associated antigen
MoAb: monoclonal antibody
SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis

MATERIALS AND METHODS

Cell Lines The G361 melanoma cell line was obtained from the Genetic Mutant Cell Repository (CRL-1424). The T 5-1 human B lymphoblastoid cell line was kindly supplied by Dr. D. Pious (University of Washington, Seattle, WA). Both cell lines were maintained in long-term tissue culture in RPMI-1640 medium (Gibco Laboratories, Grand Island, NY) containing 10% fetal bovine serum (FBS) (Gibco). The cell line SK MEL-30 was obtained from the Sloan-Kettering Cancer Center, NY, and was maintained in Eagle's minimal essential medium (Gibco) supplemented with 7.5% FBS. Serum free medium (PC-1) was purchased from Ventrex Laboratories, Inc., Portland, OR.

Monoclonal Antibody The procedures used for immunization, fusion of spleen cells with Sp2/0-Ag14 mouse myeloma cells, and screening of hybridoma clones have been described previously [2]. The subclass of MoAb HMSA-2 was IgG1, Kappa [3]. MoAb HMSA-2 was purified from ascites using Affi-Gel Protein A MAPS II kit (BioRad, Richmond, CA).

Flow Cytometry Analysis

FITC-labeling: Fluorescence analysis of cell surface and cytoplasmic antigen was carried out on an Ortho Diagnostic Systems Cytofluorograf System 50-H, using a modification of the method of Loken and Herzenberg as described [6]. G361 melanoma cells (2×10^6 cell/ml) harvested from culture in the log phase of growth were washed twice with Dulbecco's phosphate-buffered saline (DPBS) containing 0.1% sodium azide. Prior to the staining for MoAb

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HMSA-2, cells for cytoplasmic staining were fixed with 50% ethanol for 30 min. After washing 3 times with RPMI 1640 containing 10% FBS, 2 μg of purified MoAb HMSA-2 were added as a primary antibody for 60 min on ice. Culture supernatant from a mouse myeloma cell (NS-1) was used as a control antibody. Goat anti-mouse Ig labeled with fluorescein isothiocyanate (Capell) was added and incubated for 60 min on ice at a protein concentration of 50 μg/ml of antibody. Following incubation with this second antibody, the cells were washed 3 times and then analyzed in the fluorescence-activated cell sorter.

Propidium Iodide Staining: For two parameter measurements of DNA and immunofluorescence, surface stained cells were fixed in 50% ethanol for 30 min in the dark after immunofluorescence staining. The cells were washed and treated with RNase (Sigma, 200 μg/ml in DPBS) for 30 min at room temperature, centrifuged, and resuspended in propidium iodide (Sigma, 5 μg/ml). Finally, the cells were filtered through 44-μm pore size nylon mesh (Small Parts Inc., Miami, FL) for the flow cytometry analysis. The stained cells were analyzed on a System 50-H Cytofluorograf and data were recorded and analyzed by a 2150 Computer System (Ortho Instruments, Westwood, MA).

Biosynthetic Labeling G361 and T5-1 cell lines were biosynthetically labeled with ^{35}S-methionine (New England Nuclear, Boston, MA) in methionine deficient culture medium (Gibco) containing 10% FBS. After a 2-h preincubation, 500 μCi of ^{35}S-methionine was added and incubated for 18 h at 37°C. Labeling in the presence of tunicamycin (10 μg/ml in DMSO, Sigma), monensin (1 μg/ml in 95% ethanol, Sigma) was done similarly, except that both antibiotics were added to the cells 3 h prior to the addition of the ^{35}S-methionine. After labeling, the cells were washed with phosphate-buffered saline (PBS), harvested by scraping, and then incubated with 500 μl of lysis buffer [10 mM Tris-HCl, pH 7.6; 1 mM $MgCl_2$; 1% NP-40; 0.5% sodium deoxycholate; 0.1% sodium azide; 0.25% Trasylol (Sigma); 1 mM phenylmethylsulfonyl fluoride (Sigma)] for 30 min on ice. Cell lysates were centrifuged in an Eppendorf microfuge for 30 min at 4°C. Culture supernatants were centrifuged at 100,000 × g for 1 h at 4°C. Both types of samples were precleared with mouse serum agarose (Sigma) before immunoprecipitations were done. Gelatin agarose was purchased from Sigma.

Indirect Immunoprecipitation and SDS-PAGE Indirect immunoprecipitation of biosynthetically labeled antigen was carried out using purified MoAb HMSA-2 that was coupled to cyanogen bromide-activated Sepharose 4B (Pharmacia AB, Uppsala, Sweden). After the immunoadsorbent beads and cell extract or culture supernatant were allowed to react overnight at 4°C with continuous rotation, the immunoadsorbent was washed seven times with a wash buffer (0.5 M NaCl; 5 mM EDTA; 50 mM Tris-HCl; pH 7.5; 1% NP-40) and then three times with the same wash buffer with no NP-40 added. Antigens were eluted from immunoadsorbent beads by boiling with 100 μl of electrophoresis buffer for 5 min. SDS-PAGE was performed in 5% to 15% polyacrylamide slab gels [7]. Autoradiography was done by the method of Bonner and Laskey [8] using 2,5-diphenyloxazole in dimethyl sulfoxide and exposure to X-Omat K film (Eastman Kodak Co.) at −70°C. ^{14}C-labeled protein standards obtained from Amersham Canada Limited were used as molecular weight markers. These included myosin (200 kDa); phosphorlyase B (92.5 kDa), bovine serum albumin (69 kDa), ovalbumin (46 kDa), carbonic anhydrase (30 kDa), and lysozyme (14.3 kDa).

Enzymatic Digestion In this study neuraminidase *(Clostridium perfringens)* Type X (Sigma), N-glycanase (Genzyme Co., Boston, MA) and chondroitin ABC lyase (EC4.2.2.4) (Sigma) were used. Prior to analysis on SDS-PAGE, the washed immunoadsorbent beads were resuspended three times in PBS (pH 7.2) or 200 mM sodium phosphate buffer (pH 8.6), 1.25% NP-40, 0.17% SDS for neuraminidase or N-glycanase, respectively, and then boiled for 3 min. Neuraminidase (10 mU) or N-glycanase (10 U) were added to the immunoadsorbent beads and incubated at 37°C with continuous rotation overnight. Finally, the immunoadsorbent beads were washed 3 times with wash buffer and processed for SDS-PAGE analysis. In another experiment, the washed immunoadsorbent beads were incubated with sodium phosphate buffer (pH 7.6) containing 0.05 unit of chondroitin ABC lyase. After a 1-h incubation at 37°C with continuous rotation, the antigens were eluted with sample buffer and were analyzed by SDS-PAGE. In each case the control was treated similarly, except that neuraminidase, N-glycanase, or chondroitin ABC lyase, respectively, were omitted.

Immunoelectron Microscopic Study Details of this method have been reported [9]. Briefly, SK MEL-30 melanoma cells were grown in tissue culture chambers (Lab Tek, U.S.A.). They were prefixed with 4% paraformaldehyde in 0.1 M PBS for 1 h at room temperature, and then treated with 0.2% saponin for 30 min. Hybridoma culture supernatants containing MoAb HMSA-2 supplemented with 0.02% saponin were used as a primary antibody. After washing, immunostaining was carried out with avidin-biotin-peroxidase complex (Vector Laboratories, Inc., Burlingame, CA). Finally, the cells were postfixed with 1% OsO_4 cacodylate buffer solution, dehydrated, and embedded in epon.

Enzyme-Linked Immunosorbent Assay (ELISA) After centrifuging the G361 culture supernatants containing RPMI 1640 with 10% FBS or serum free medium (PC-1) at 100,000 × g for 1 h, ammonium sulfate was added to the supernatants to make a 70% (w/v) solution, and this was stirred for 30 min at room temperature. After centrifugation at 3,000 × g, the pellets were dissolved and dialyzed extensively against PBS. 0.15 M iodoacetic acid and 1 mM PMSF were added, and the supernatants thus prepared were used as the antigen coating for ELISA. The method for the ELISA has been reported previously [10]. The concentration of purified MoAb HMSA-2 was 10 μg/ml. A horse radish peroxidase-conjugated goat anti-mouse Ig (Kirkegaard & Perry Laboratories, Inc.) was used as a second antibody. The peroxidase reaction used the substrate 2,2′-azino-di-[3-ethyl-benzthiazoline sulfonate]. The reactions were read on a Multiscan spectrophotometer (Flow, Inglewood, CA).

RESULTS

The fluorescence histogram patterns of reactivity of MoAb HMSA-2 are illustrated in Fig 1*A,B*. The MoAb recognized cytoplasmic antigens in 60%-80% of the total cell population of G361 melanoma cells, and surface antigens on 10% – 20% of the cells. The fluorescence intensity of the cytoplasmic antigens was also stronger. It appeared that HMSA-2 antigen was expressed mainly in the cytoplasm of the cultured human melanoma cell lines, and less so on the cell surface.

Two-color flow cytometric analysis of cytoplasmic expression of MoAb HMSA-2 showed that the mean cell volume increased during the progression of the cell cycle, and that fluorescence intensity correlated with cell volume; i.e., HMSA-2 was maximally detected during the G2 + M phase. Surface expression of HMSA-2 was comparatively stable throughout the cell cycle, although the antibody bound maximally during S phase (Table I).

Indirect immunoprecipitation of culture supernatant from ^{35}S-methionine labeled G361 melanoma cells with MoAb HMSA-2 in SDS-PAGE revealed three major glycoproteins with molecular weights of 250, 94, and 53 kDa, and a minor glycoprotein of 205 kDa (Figs 2 and 3). The 53-kDa glycoprotein recognized by MoAb HMSA-2 in this study is in agreement with that reported previously [2]. When immunoprecipitations were carried out on ^{35}S-methionine labeled whole cell lysates of G361 cells, only very weak bands corresponding to the HMSA-2 components described above were seen on SDS-PAGE gels (data not shown).

The proteoglycan-specific enzyme chondroitin ABC lyase showed no apparent effect on the 250-kDa component in the affinity precipitation produced with gelatin agarose. Moreover, the 250-kDa component in the final HMSA-2 immunoprecipitation decreased or disappeared upon preclearing with gelatin agarose (Fig 4). These observations suggest that the 250-kDa component was

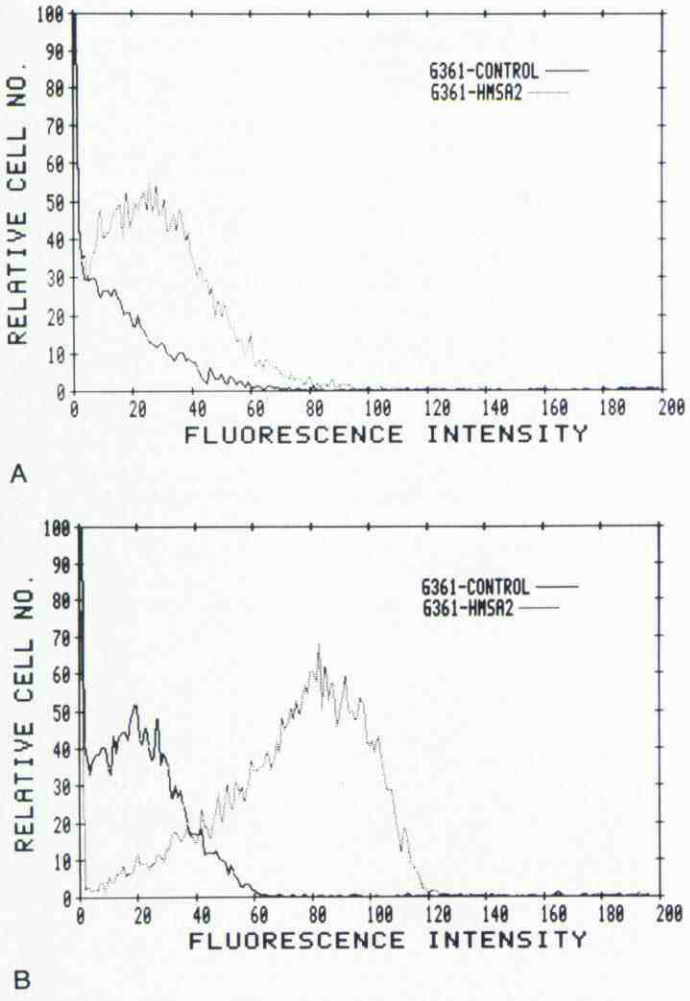


Figure 1. Fluorescence histograms of G361 melanoma cells stained with MoAb HMSA-2 or control. *A:* Surface staining with MoAb HMSA-2 *(dashed line)* or control *(solid line)*. *B:* Cytoplasmic staining with MoAb HMSA-2 *(dashed line)* or control *(solid line)*. The antigen recognized by MoAb HMSA-2 is expressed mainly in the cytoplasm with less detected on the cell surface.

fibronectin. It is unclear whether it is melanoma specific or not. After treatment with neuraminidase the 94- and 53-kDa components were processed into a sharp 83-kDa band and a 56-kDa heterogeneous band, respectively (Fig 3). When the immunosorbent beads were treated with N-glycanase, which hydrolyses the glycosylamine linkage of all types of asparagine-linked oligosaccharides, the 94- and 53-kDa components disappeared, while the intermediate, partially trimmed glycoprotein could be detected at 65, 60 and 35 kDa (Fig 3). When G361 cells were labeled in the presence of tunicamycin, which inhibits the synthesis of N-linked oligosaccharide side chains, the 94- and 53-kDa components, which had been detected in the culture supernatant, were no longer recognized

Table I. Flow Cytometry Analysis of Indirect Immunofluorescence of G361 Melanoma Cells in Relation to Position in Cell Cycle and Cell Size

	G0/G1	S	G2 + M
Surface			
% cells in phase	39.5	32.7	27.9
% FITC-MoAb HMSA-2 positive	7.0	13.3	10.8
Mean fluorescence	248.7	362.9	343.3
Cell size (μ)	20.91	22.24	24.70
Cytoplasmic			
% cells in phase	37.4	39.5	23.1
% FITC-MoAb HMSA-2 positive	34.8	51.4	68.2
Mean fluorescence	395.9	405.0	446.7
Cell size (μ)	20.82	21.82	25.44

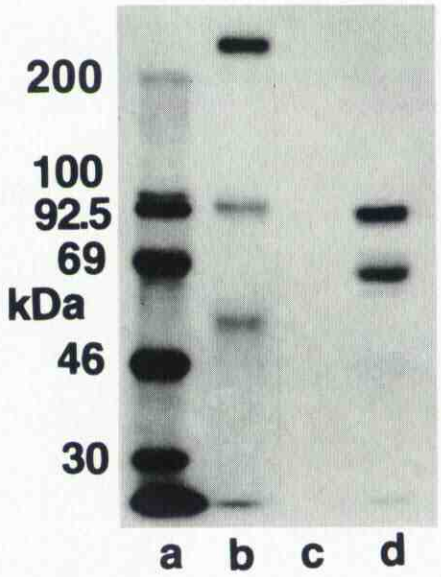


Figure 2. Fluorograph obtained by immunoprecipitating G361 melanoma culture supernatant, biosynthetically labeled with ^{35}S-methionine with MoAb HMSA-2. *a:* Molecular weight standards. *b:* Immunoprecipitation of G361 melanoma culture supernatant with MoAb HMSA-2. *c:* Control (using T5-1 B-cell line). *d:* Control (using normal serum agarose).

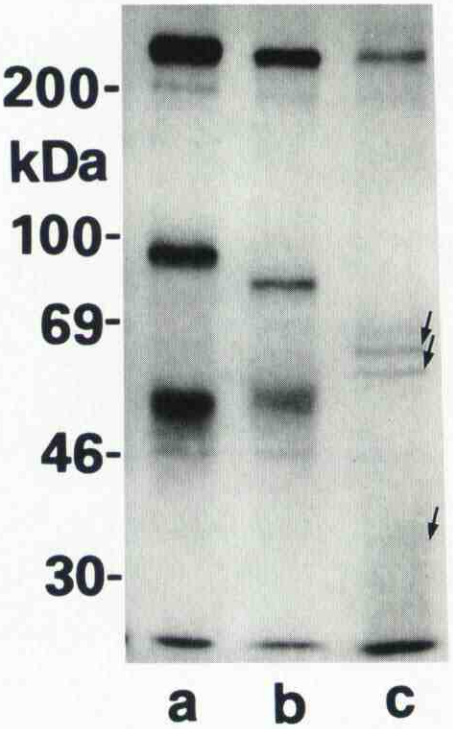


Figure 3. Fluorograph obtained by immunoprecipitating G361 melanoma culture supernatant after treatment with neuraminidase and N-glycanase. *a:* No enzyme treatments. *b:* Neuraminidase treatment. *c:* N-glycanase treatment. *Arrows* indicate 65, 60, and 35 kDa, respectively.

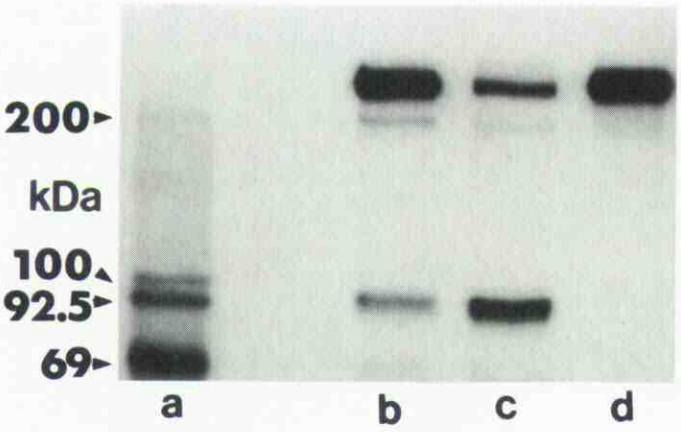


Figure 4. Fluorograph obtained by immunoprecipitating G361 melanoma culture supernatant after preclearing with gelatin agarose. *a:* Molecular weight standards. *b:* Immunoprecipitation of G361 melanoma culture supernatant with MoAb HMSA-2. *c:* Immunoprecipitation of G361 melanoma culture supernatant with MoAb HMSA-2 after preclearing with gelatin agarose. *d:* Immunoprecipitation of G361 melanoma culture supernatant with gelatin agarose. The 250-kDa component was decreased by preclearing with gelatin agarose. These observations suggest that the 250-kDa component is fibronectin.

by MoAb HMSA-2. Instead, two new faint specific bands of 60 and 35 kDa appeared (Fig 5). The additional 46-kDa component is believed to be actin. When G361 cells were labeled in the presence of monensin, which can inhibit cell surface expression of membrane glycoproteins by blocking vesicle traffic in the Golgi, the 94-kDa component was processed into an 83-kDa band. This had the same molecular weight as had been seen with neuraminidase treatment (Figs 3 and 5). Although neuraminidase and N-glycanase treatments had no effect on the 205-kDa component, it disappeared after both tunicamycin and monensin treatment. Under nonreducing conditions the 94-kDa glycoprotein actually migrates somewhat faster, suggesting that it consists of a single chain possessing some intrachain disulfide bonding (data not shown).

Immunoelectron microscopy using the saponin permeation method showed that MoAb HMSA-2 localized on the vacuolar structures of stage I melanosomes and on the inner matrix of stage II or III melanosomes. The nucleus, mitochondria, Golgi complex, endoplasmic reticulum, and stage IV melanosomes did not react with MoAb HMSA-2. Moreover, no reactivity of the plasma membrane was observed by immunoelectron microscopy, despite the positive results with flow cytometry (Fig 6). There are two possible explanations for this discrepancy: one is that saponin treatment may destroy the membrane structure with subsequent loss of the reactivity, and the other is that the peroxidase-diaminobenzidine reaction products may diffuse during the fixation procedure. These observations are similar to those obtained previously with MoAb HMSA-1 [9]. Thus the MoAb HMSA-2 appears to recognize a melanosomal matrix structure, but not tyrosinase, in a similar manner to MoAb HMSA-1.

In Fig 7, serial dilutions of G361 culture supernatants containing HMSA-2 antigen (starting material: 1.2 mg/ml) were fixed on microtiter plates for the ELISA using the MoAb HMSA-2. The degree

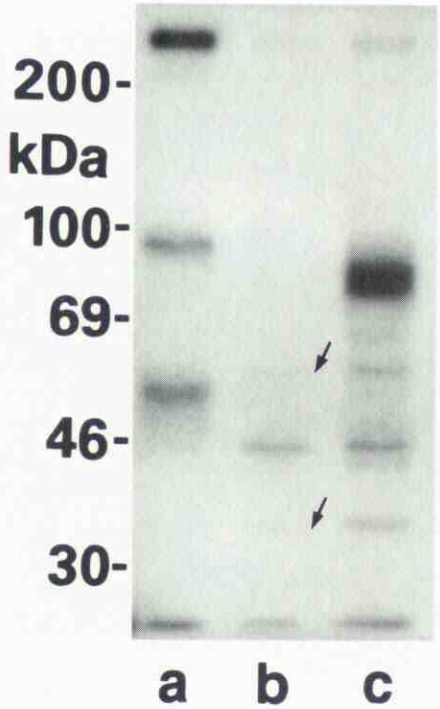
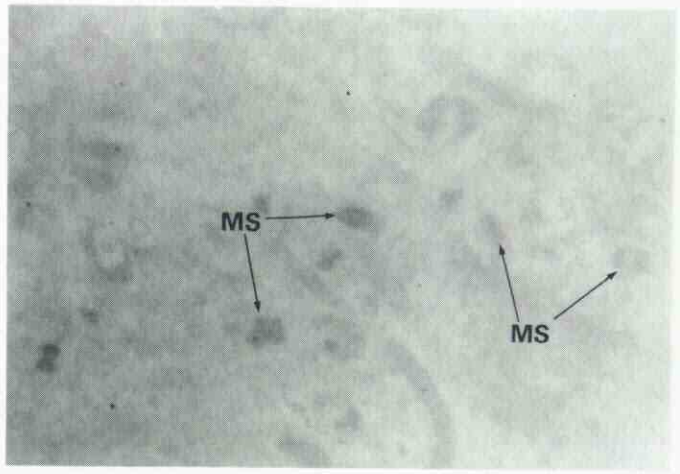


Figure 5. Fluorograph obtained by immunoprecipitating G361 melanoma culture supernatant after treatment with tunicamycin and monensin. *a:* No antibiotics treatments. *b:* Tunicamycin treatment. *c:* Monensin treatment. *Arrows* indicate 60 and 35 kDa, respectively.



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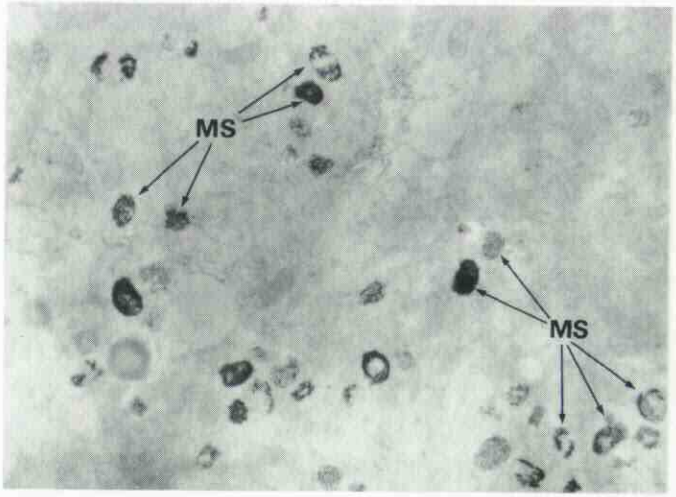

B

Figure 6. Immunoelectron microscopic study on the SK MEL-30 melanoma cell line with MoAb HMSA-2 using the saponin permeation method. *A:* Control × 14,000 *B:* HMSA-2 × 14,000. Reaction products of MoAb HMSA-2 are present in the melanosomes *(MS)*.

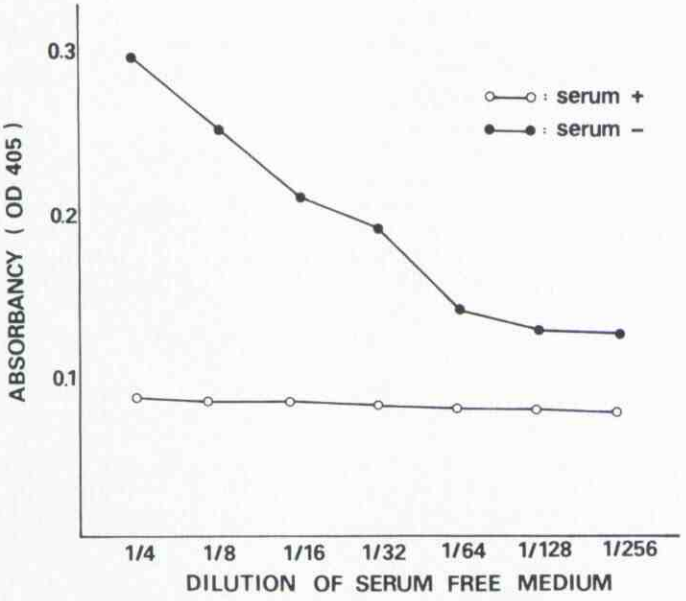

Figure 7. The relation of reactivity to MoAb HMSA-2 in ELISA and the protein concentration of shed antigen in culture supernatant. Serial dilutions of sample (starting material: 1.2 mg/ml) were fixed on microtiter plates. *Open circles:* RPMI 1640 containing 10% FBS. *Closed circles:* Serum free medium (PC-1).

of the reactivity of shed HMSA in serum-free medium linearly decreased with the dilution, whereas that of shed HMSA-2 antigen in RPMI 1640 containing 10% FBS medium showed no decline with dilution. Thus the ELISA method can detect HMSA shed into culture supernatants in serum free media, but a high concentration of FBS in the medium may interfere with the fixation of antigens to the microtiter plate.

DISCUSSION

MoAb HMSA-2 was raised against partially purified melanosomal protein [2]. The present study has shown that a) The antigens recognized by MoAb HMSA-2 are melanosomal matrix glycoproteins of various molecular weights. They are expressed mainly in the cytoplasm, although some melanoma cells have a low level of expression on the cell surface. The cytoplasmic expression of the HMSA-2 antigen appears to be cell-cycle dependent. b) The HMSA-2 reactive antigens can be detected in the culture supernatant and consist of 94- and 53-kDa species, which are characterized by the presence of complex N-linked oligosaccharide chains with sialic acids. The 94-kDa component also possesses some intrachain disulfide bonding, as revealed by its more rapid migration on SDS-PAGE gels run under non-reducing conditions. c) The antigens reactive with MoAb HMSA-2 are localized in stage I melanosomes and on the inner matrix of stage II or III melanosomes, but not stage IV ones.

In previous studies, antigenic heterogeneity in the cytoplasm of malignant melanoma cells stained with MoAb HMSA-2 was reported [2,4]. Our immunoelectron microscopic findings provide an explanation in that nonpigmented or weakly pigmented melanomas reacted much more intensely with MoAb HMSA-2 than did heavily pigmented ones. Furthermore, we have observed that cytoplasmic antigens recognized by MoAb HMSA-2 are expressed mainly in the G2 + M phase of the cell cycle. Thus heterogeneous expression of HMSA-2 within the same pathologic lesion may be due also to cell-cycle status.

Indirect immunoprecipitation of culture supernatant with MoAb HMSA-2 revealed three major glycoproteins of 250, 94, and 53 kDa, as well as a minor glycoprotein of 205 kDa. The 250-kDa component appears to be a fibronectin-related molecule, because it can be removed by preclearing prior to immunoprecipitation with gelatin agarose. The immunohistochemical profile of MoAb HMSA-2 is very different from that of anti-fibronectin monoclonal antibodies. Fibronectin is a large glycoprotein and is secreted into the culture media of substrate-attached cells and deposited in the extracellular connective tissue matrix [11]. It is possible that MoAb HMSA-2 may react with a fibronectin-melanosomal glycoprotein complex, but it could not be determined in this study whether the 250-kDa component was melanoma specific (melanoma-fibronectin) or not.

Recently, several monoclonal antibodies recognizing high molecular weight melanoma associated antigens (HMW-MAA) have been described by several groups. Among these antibodies, 9.2.27 [12], 155.8 [13], 48.7 [14] and O_1-95-45 [15] have been shown to react with a glycoprotein of about 250 kDa. These monoclonals appear to recognize the same antigen or different epitopes on the same antigen. However, the tissue distribution and structure of the antigens recognized by MoAb HMSA-2 are different.

To study the biochemical characteristics of the antigens recognized by MoAb HMSA-2, we used several antibiotics and enzymes. Tunicamycin, an analog of UDP-N-acetylglucosamine, interferes with the formation of the N-acetylglucosamine pyrophosphoryldolichol intermediate and completely inhibits the synthesis of N-linked oligosaccharide side chains. N-glycanase can remove N-linked oligosaccharide side-chains. Monensin inhibits protein transport from the medial to the trans Golgi cisternae, and thereby blocks the trimming of high-mannose oligosaccharides bound to proteins as well as their conversion to complex oligosaccharides [16]. Our results using these reagents indicate that the 94-kDa component possessed complex N-linked oligosaccharide chains with sialic acids. This glycosylation pattern may reflect the biosynthetic origin and cellular destination of a particular organelle and its constituents [20]. N-linked oligosaccharides are modified by the action of N-acetylglucosaminyl transferase I in the medial Golgi, and subsequent additions of galactose and sialic acid occur in the trans Golgi [17]. Thus the 94-kDa melanosomal glycoprotein is likely derived from the trans Golgi component. In cells treated with monensin, the 94-kDa component was replaced by an 83-kDa band, identical to that seen upon in vitro treatment of HMSA-2 reactive antigen with neuraminidase. These observations suggest the existence of a route that transports melanosomal antigens from cis or medial Golgi to the cell surface or extracellularly, bypassing the trans Golgi.

The 53-kDa glycoprotein also possessed complex N-linked oligosaccharide chains with sialic acids. In contrast to the 94-kDa glycoprotein, however, this 53-kDa component migrates somewhat slower at 56 kDa after treatment with neuraminidase. This observation suggests that the 53-kDa component contains distal sialic acids. In general, it is believed that removal of the distal sialic acids from O-glycosidic oligosaccharides increases the apparent molecular weights of polypeptides containing O-linked carbohydrate chains, evidently due to the decreased total ratio of charge to mass [18].

There are several hypotheses concerning how structural proteins and tyrosinase are organized to form early melanosomes [19]. The best accepted explanation is that the site of incorporation of structural protein and tyrosinase into melanosomes is different [20]. Recently, Roux and Lloyd [21,22] reported the biochemical characteristics of pigmentation-associated antigen (PAA) recognized by monoclonal antibody TA99. PAA is a melanosomal glycoprotein and a structural component of melanosomes. The main glycosylation features of PAA are N-linked complex carbohydrates with high mannose content. This indicates that PAA is derived from the trans portion of Golgi stacks, because it has sialylated complex chains which would need to pass through this portion of the GERL to be synthesized [21]. The antigen recognized by MoAb HMSA-2 is in immature melanosomes, whereas PAA is confined to a subpopulation of mature melanosomes. However, our data also indicate that HMSA-2 is derived from trans Golgi. Some kinds of melanosomes or melanosomal proteins are assembled in the trans Golgi, but not in the sER.

Several monoclonal antibodies have been reported to identify a 94-kDa component in human melanoma cells. MoAb 465.12 did

not recognize melanosome-related antigens and did not react with formalin-fixed and paraffin-processed sections [23,24]. Gp95 [25], p97 [26], and HMB-50 [27] are also quite different in their properties from MoAb HMSA-2. Interestingly, the antigenic structures reacting with MoAb HMSA-2 are very similar to those identified by the polyclonal anti-melanoma xenoantisera, 6522 [28] and 8986 [29]. Both xenoantisera reacted with 240- and 94-kDa glycoproteins. The 240-kDa glycoprotein was present only on melanoma cells, whereas the 94-kDa glycoprotein was also found on fetal melanocytes and on carcinoma cells. These antigens were detected in culture supernatant. In contrast to MoAb HMSA-2, however, these two xenoantisera reacted with cell surface antigens and did not recognize any 53-kDa glycoprotein components.

Thus MoAb HMSA-2 recognizes antigens different from those already described in the literature. Large amounts of the antigens recognized by MoAb HMSA-2 are shed into the culture supernatant in vitro, and it may be that these antigens are released into the body fluids of tumor-bearing patients. Although the precise function of the HMSA-2 reactive antigen(s) is not understood, it does appear to be a structural component of the melanosome, and its stage-specific nature may suggest a role in the maturation of melanosomes. In conclusion, MoAb HMSA-2 may become a useful diagnostic tool, not only for immunohistochemical studies, but also for serologic immunodiagnosis. In addition, MoAb HMSA-2 may also be useful for studies investigating the immunobiology of melanogenesis.

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